adults [**2B**]

7.4 Boys and girls should generally be treated at similar ages, although with a particularly adverse family history of CHD and other major risk factors, boys with heterozygous FH could be considered for earlier treatment with statins. [**2B**]

7.5 Children, between the ages of 8 and 10 years, with proven FH on a suitable diet and LDL-cholesterol >4.0 mmol/L on two occasions should be started on low-dose statin monotherapy, aiming for an LDL-cholesterol <4.0 mmol/L. [**3C**]

7.6 After the age of 10 years, children with proven FH on a suitable diet and LDL-cholesterol >3.5 mmol/L on two occasions should be started on statin monotherapy, aiming for an LDL-cholesterol <3.5 mmol/L, with the addition of ezetimibe or a bile acid sequestrant if required. [**3C**]

7.7 The preferred statins for initiating therapy are those that are licensed for clinical use in this age group in specific countries; other statins may be prescribed according to clinical indications, higher doses of potent statins being required in homozygotes. [**1C**]

7.8 Although statins can be safely used in children, weight, growth, physical and sexual development, and well-being should be monitored in this age group. [**1A**]

7.9 Plasma levels of hepatic aminotransferases, creatine kinase, glucose and creatinine should be measured before starting drug therapy. All patients on statins should have hepatic aminotransferases monitored; creatine kinase should be measured and compared to pre-treatment levels when musculoskeletal symptoms are reported; glucose should be monitored if there are risk factors for diabetes. [**2A**]

7.10 All adolescent girls should receive pre-pregnancy counselling, with appropriate advice on contraception, before starting a statin and this should be reinforced at annual review. [**3A**].

7.11 Although carotid ultrasonography has been used in clinical trials, its role in monitoring therapy in patients with heterozygous FH has not been established and it should therefore not be used for this purpose. [**3C**]

7.12 Well controlled and lower complexity patients should be followed up in primary care, whereas higher complexity patients will need regular review by a paediatrician. Opportunities should be created for integrated care between GPs and paediatricians. Family based and transitional care clinics should be considered by adult and paediatric services. [**3B**]

7.13 Children with homozygous FH should be referred on diagnosis to a specialist centre and drug and/or apheresis treatment commenced as soon as possible. [**2A**]

7.14 In children with homozygous FH and rapidly progressive atherosclerosis, Lomitapide and Mipomersen, although not yet tested in children, should be considered, employing special access or compassionate use schemes, as adjunctive treatments to diet and conventional drugs to further reduce plasma LDL-cholesterol, particularly if apheresis is not available or declined by the patient/family. [**3C**]

8. Lipoprotein apheresis and related treatments

8.1 Lipoprotein apheresis (LA) should be considered in all patients with homozygous or compound heterozygous FH (i.e. homozygous FH phenotype) and carried out in a dedicated centre with the relevant expertise. [**1A**]

8.2 LA should be considered in patients with heterozygous FH with CHD who cannot achieve LDL-cholesterol targets despite maximal drug therapy or because they cannot tolerate statins. [**2A**]

8.3 LA should be considered in children with homozygous FH by the age of 5 and no later than 8 years. [**2A**]

8.4 Diet and drug therapy to lower LDL-cholesterol should be continued during treatment with LA [**2A**].

8.5 The efficacy, tolerability and safety of LA must be regularly reviewed. [**3A**]

8.6 The effect of LA on progression of atherosclerosis should be monitored according to clinical indications in FH patients with echocardiography (aortic valve and root), carotid ultrasonography and exercise stress testing. [**3B**]

8.7 Lomitapide should be considered as an adjunctive to standard diet and drug therapy to further lower plasma LDL-cholesterol in adults with homozygous FH on LA. [**1C**]

8.8 Lomitapide should be considered, via a special access scheme, as an adjunctive treatment to further lower plasma LDL-cholesterol in children and adolescents with homozygous FH on LA with rapidly progressive atherosclerosis. [**3C**]

8.9 Mipomersen should be considered as an adjunctive to standard diet and drug therapy to further lower plasma LDL-cholesterol in adults, children and adolescents with homozygous FH on LA who cannot tolerate lomitapide. [**3C**]

8.10 If available, orthotopic liver transplantation should be considered for younger patients with homozygous FH who have rapid progression of atherosclerosis or aortic stenosis, cannot tolerate LA or when plasma LDL-cholesterol cannot be adequately lowered with LA, diet and drug treatment. [**3B**]

9. Organization and Development of Care

9.1 Care pathways for FH should be developed for country-specific and local needs. [**3A**]

9.2 Specialist services should be multidisciplinary based and integrated with primary care. [**3B**]

9.3 Specialist care of FH should ideally be supported by cardiology, paediatric, genetic, imaging, transfusion medicine, nursing, dietetic, psychology, pharmacy and pathology laboratory services. [**3A**]

9.4 Cascade screening should ideally be centrally co-ordinated by a dedicated centre. [**1A**]

9.5 Low complexity patients should be managed in primary care, with the option of annual specialist review. [**3A**]

9.6 Higher complexity patients should be managed principally in specialist centres. [**3A**]

9.7 Medical, nursing and allied health staff managing patients with FH should be accredited in cardiovascular prevention. [**3A**]

9.8 Services should establish partnerships with academic and professional organizations to enhance teaching, training and research. [**3A**]

9.9 A registry of patients and families should be established for clinical, research and audit purposes. [**3A**]

9.10 A support group of patients and families should be established as a major priority for enhancing public, government and health care provider awareness, as well as the total quality of care of FH. [**3A**]

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Impact of the Integrated Guidance on the Care of Familial Hypercholesterolaemia

Watts G.F. *et al.* recently reported the Integrated Guidance on the Care of Familial Hypercholesterolaemia issued by the International FH Foundation[1]. On behalf of the Asia-Pacific Society of Atherosclerosis and Vascular Biology (APSAVD), we herein describe our perspective regarding the care of FH. In this summary, the authors described the guidelines for the detection, diagnosis, assessment and management of familial hypercholesterolemia (FH) in adults and children, which were determined following the discussions held at seminars and workshops at the 16th International Symposium on Atherosclerosis in Sydney in 2012. The recommended treatment is based on risk stratification, the management of non-cholesterol risk factors and the administration of safe and effective treatment to lower the LDL-cholesterol level. In addition to treatment with lipid-lowering medications, such as statins, ezetimibe, resin, fibrates and probucol, the authors described emerging therapies for FH, including mipomersen, lomitapide and anti-PCSK9 antibodies. These guidelines should have a significant impact on the management of FH in the Asia-Pacific region, as awareness of the clinical importance of FH remains very low in many countries, despite the fact that more than half of the world's population lives in this region. In the Asia-Pacific region, Japan and Australia are the only countries with published guidelines in English for the diagnosis and management of FH[2, 3], and only a few countries have published such guidelines in their mother language. FH is an autosomal dominant disease caused by the presence of abnormal LDL receptors or LDL receptor-related genes that is characterized by the triad of hyper-LDL-cholesterolemia, premature coronary artery disease (CAD) and tendon/cutaneous xanthoma. In our Japanese guidelines, we revised our diagnostic criteria for heterozygous FH, as indicated in **Table 1**, in a somewhat similar fashion to Simon Broome's criteria, although we determined the cutoff value for the LDL-cholesterol level based on the results of our multicenter study[4]. Considering that FH by itself is a very high-risk condition for CAD and that untreated patients are likely to develop CAD, such as myocardial infarction and angina pectoris, at a young age[3], providing an early diagnosis and appropriate treatment is mandatory for preventing premature death. Additionally, heterozygous FH is detected in one in 137 to 500 individuals and is one of the most frequently encountered genetic diseases in general practice[5]. Therefore, according to these guidelines, it is important to encourage the training of specialists of FH in each country and educate general practitioners regarding the diagnosis and treatment of FH. We hope that these guidelines will help to spread knowledge regarding the clinical implications of FH throughout the Asia-Pacific region and identify gaps in care, including collaborative efforts to enhance detection (especially in children), the introduction of effective early treatment, the development of country-specific models of care and the establishment of family support groups, relevant research agendas and funding mechanisms by the government and other organizations.

Table 1. Diagnostic Criteria for Heterozygous FH in Adults (Aged 15 Years or Older)[2]

1. Hyper-LDL-cholesterolemia (untreated LDL-C of ≥ 180 mg/dL)
2. Tendon xanthoma (tendon xanthoma on the backs of hands, elbows, knees, etc. or Achilles tendon hypertrophy) or xanthoma tuberosum
3. Family history of FH or premature CAD (within the second-degree relatives)

- Diagnosis should be made after excluding secondary hyperlipidemia
- If a patient meets two or three of the above-mentioned criteria, the condition should be diagnosed as FH. In the case of suspected FH, diagnosis by genetic testing is desirable.
- Xanthoma palpebrarum is not included in xanthoma tuberosum.
- Achilles tendon hypertrophy is diagnosed if the Achilles tendon thickness is ≥ 9 mm on soft X-ray imaging.
- An LDL-C of ≥ 250 mg/dL strongly suggests FH.
- If a patient is already receiving drug therapy, the lipid level that led to treatment should be used as the reference for diagnosis.
- Premature CAD is defined as CAD in men aged < 55 years or women aged < 65 years.
- If FH is diagnosed, it is preferable to also examine the patient's family members.

Conflicts of Interest

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Executive Summary

Integrated Guidance on the Care of Familial Hypercholesterolaemia from the International FH Foundation: Executive Summary

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Familial hypercholesterolaemia (FH) is a dominantly inherited disorder present from birth that markedly elevates plasma low-density lipoprotein (LDL) cholesterol and causes premature coronary heart disease. There are at least 20 million people with FH worldwide, but the majority remains undetected and current treatment is often suboptimal.
To address this major gap in coronary prevention we present, from an international perspective, consensus-based guidance on the care of FH. The guidance was generated from seminars and workshops held at an international symposium. The recommendations focus on the detection, diagnosis, assessment and management of FH in adults and children, and set guidelines for clinical purposes. They also refer to best practice for cascade screening and risk notifying and testing families for FH, including use of genetic testing. Guidance on treatment is based on risk stratification, management of non-cholesterol risk factors and safe and effective use of LDL lowering therapies. Recommendations are given on lipoprotein apheresis. The use of emerging therapies for FH is also foreshadowed.
This international guidance acknowledges evidence gaps, but aims to make the best use of contemporary practice and technology to achieve the best outcomes for the care of FH. It should accordingly be employed to inform clinical judgment and be adjusted for country-specific and local healthcare needs and resources.

Key words: Familial hypercholesterolaemia, International guidance, Adults, Children, Screening, Diagnosis, Assessment, Treatment, Models of care

Endorsement

The recommendations contained in this document have been fully endorsed by The National Lipid Association, 6816 Southpoint Parkway (Suite 1000), Jacksonville, FL3316, US.

Conversion Factor

mg/dL cholesterol = mmol/L × 38.7

Levels of Evidence and Grades of Recommendation

Levels of Evidence

1 = systematic review/meta-analysis/at least one randomised control trial/good quality diagnostic tests.

2 = good quality clinical or observational studies.

3 = expert opinion or clinical experience/argument from first principles.

(The evidence for therapeutic interventions was considered principally in respect of effects on plasma LDL-cholesterol concentrations, but where available was also based on data on subclinical atherosclerosis or cardiovascular outcomes.)

Grades of Recommendation

A = can be trusted to guide practice.

B = can be trusted to guide practice in most situations.

C = can be used to guide practice, but care should be taken in application.

Summary of Recommendations

1. Detection of Index Cases: Screening and Phenotypic Diagnosis

1.1 Targeted, opportunistic and universal screening strategies should be employed to detect index cases [**2B**].

1.2 Index cases should be sought by targeted screening of adults with premature cardiovascular disease (CVD), primarily coronary heart disease (CHD) and a personal and/or family history of hypercholesterolaemia. [**1A**]

1.3 Opportunistic screening of adults and children in primary care, based on age- and gender-specific plasma LDL-cholesterol levels, should be routinely adopted. [**2B**]

1.4 Universal screening based on age- and gender- specific plasma LDL-cholesterol levels should be considered prior to age 20 years and ideally before puberty. [**2C**]

1.5 In adults, country-specific clinical tools, such as the Dutch Lipid Clinic Network, Simon Broome, MED-PED or Japanese FH criteria, may be used to make a phenotypic diagnosis. [**1A**]

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(Watts G.F. et al: Int J Cardiol, 2014; 171: 309-325)

1.6 The effect of acute illness and concurrent use of statins in lowering plasma LDL-cholesterol must be considered: testing for FH should not be carried out during acute illness; LDL-cholesterol level should be appropriately adjusted in people on statins, particularly if a reliable pre-treatment value is not available [**2A**]

1.7 All patients with suspected FH should be referred to a clinic specialising in lipidology and/or metabolic disorders for further assessment, if such a service is available. [**3A**]

2. Diagnosis and Assessment of Adults

2.1 Secondary causes of hypercholesterolaemia should first be excluded. [**1A**]

2.2 The most reliable diagnosis of FH can be made using both phenotypic (see 1.5 above and 4.8 below) criteria and genetic testing, but when genetic testing is not available the diagnosis can be made phenotypically. [**1A**]

2.3 DNA testing increases the accuracy of detecting FH and, if resources permit, should be considered to confirm the diagnosis, especially if cascade screening is planned; a fully accredited laboratory should be used. [**1A**]

2.4 Although FH is a life-time coronary risk equivalent, patients should be assessed for additional major cardiovascular risk factors, including lipoprotein(a) [Lp(a)], the level of hypercholesterolaemia at diagnosis and the prematurity of the family (especially first-degree relatives) or personal history of CVD. Framingham or other cardiovascular risk equations should not be used. [**2A**]

2.5 The presence of additional cardiovascular risk factors should guide the intensity of medical management. [**2A**]

2.6 Cardiovascular imaging (eg. cardiac computed tomography and carotid ultrasonography) may be useful for assessing asymptomatic patients, but its value is not fully established. [**2C**]

3. Diagnosis and Assessment of Children and Adolescents

3.1 Secondary causes of hypercholesterolaemia should first be excluded. [**1A**]

3.2 With the exceptions noted in 3.3, children should be genetically tested for FH only after a pathogenic variant (mutation) has been identified in a parent or first degree relative. [**1A**]

3.3 Children may initially be genetically tested for FH when parents or first degree relatives are unknown or deceased, or as an accepted screening practice in certain countries, such as the Netherlands [**3B**]

3.4 Age-, gender- and country -specific plasma LDL-cholesterol concentration thresholds should be used to make the phenotypic diagnosis; because of biological variation, two fasting LDL-cholesterol values are recommended. [**1B**]

3.5 A plasma LDL-cholesterol of 5.0 mmol/L or above indicates high probability of FH in the absence of a positive parental history of hypercholesterolaemia or premature CHD; an LDL-cholesterol of 4.0 mmol/L or above indicates high probability of FH in the presence of a positive parental history of hypercholesterolaemia or premature CHD [**1B**]

3.6 Patients should be risk stratified according to age, presence of other cardiovascular risk factors, family history of early onset CVD (especially in first-degree relatives) and the level of LDL-cholesterol at diagnosis. [**2A**]

3.7 The presence of additional cardiovascular risk factors, and hence risk stratification, should guide the intensity of medical management. [**3A**]

3.8 Carotid ultrasonography may be employed to assess risk, but its value is not fully established; it should only be carried out in centres with specific expertise. [**2C**]

3.9 Cardiac CT should not be used routinely to assess patients with heterozygous FH. [**3A**]

4. Cascade Screening: Testing and Risk Notification of Families

4.1 Notification of relatives at risk of FH should generally not be carried out without the consent of the index case. [**3A**]

4.2 Relatives should only be directly notified of their risk without consent of the index case if there is specific legislative provision for breach of confidentiality in the relevant jurisdiction. [**3C**]

4.3 A proactive approach that respects the principles of privacy, justice and autonomy is required. [**3A**]

4.4 Pre-testing counselling should be offered to at risk family members of an index case prior to any form of testing. [**1A**]

4.5 Systematic cascade screening should ideally be co-ordinated by a dedicated centre and should not be carried out in primary care without central co-ordination, particularly if employing DNA testing. [**1B**]

4.6 Cascade screening of families should be carried out using both a phenotypic and genotypic strategy, but if DNA testing is not available a phenotypic strategy alone should be used [**1A**]

4.7 Cascade screening should initially be carried out as a priority in first-degree relatives and then extended to second- and third-degree relatives. [**1A**]

4.8 In the absence of genetic testing, the diagno-

sis of FH should be made in close relatives using age- , gender- and country- specific plasma LDL-cholesterol levels. Diagnostic clinical tools for index cases, such as the Dutch Lipid Clinic Network and Simon Broome criteria, should not be employed to make the diagnosis of FH in relatives [**1A**]

4.9 DNA testing makes cascade screening more cost-effective and should be employed to screen family members after the mutation is identified in the index case. [**1A**]

4.10 Children with xanthomata or other physical findings of homozygous FH, or at risk of homozygous FH should be screened as early as possible and definitely by 2 years of age. [**2A**]

4.11 Children with suspected heterozygous FH should be screened between the ages of 5 and 10 years; age at screening should be similar in boys and girls. [**2B**]

5. Genetic Testing

5.1 Genetic testing for FH should ideally be offered to all 'index cases' who have a phenotypic diagnosis of FH. [**3A**]

5.2 When the phenotypic diagnosis of FH is unlikely (e.g. by Dutch Lipid Clinic Network Criteria), genetic testing of the 'index case' need not be carried out. [**1C**]

5.3 Genetic testing for FH must be carried out in an accredited laboratory using standardised methods that target specific mutations and/or by exon-by-exon sequencing. [**1A**]

5.4 If genetic testing detects a variant, its significance as a pathogenic mutation, a previously reported variant of uncertain significance, a novel variant of uncertain significance or a benign (normal) variant needs to be assessed and recorded. [**1A**]

5.5 If genetic testing does not detect a variant, FH due to undetected mutations or mutations in untested genes cannot be excluded, particularly if the clinical phenotype is strongly suggestive of FH. [**1A**]

6. Management of Adults

6.1 All adult patients with FH must receive advice on lifestyle modifications and advice to correct all non-cholesterol risk factors should be provided according to expert recommendations. [**2A**]

6.2 Therapy should ideally aim for at least a 50% reduction in plasma LDL-cholesterol, followed by an LDL-cholesterol <2.5 mmol/L (absence of CHD or other major risk factors) and <1.8 mmol/L (presence of CHD or other major risk factors). [**2C**]

6.3 Achieving these targets will require a fat-modified, heart-healthy diet and statin therapy with or without ezetimibe. [**1A**]

6.4 Drug combinations including bile acid sequestrants, niacin, probucol or fibrates, may be required with more intensive strategies to further reduce LDL-cholesterol. [**1B**]

6.5 Plasma levels of hepatic aminotransferases, creatine kinase, glucose and creatinine should be measured before starting drug therapy. All patients on statins should have hepatic aminotransferases monitored; creatine kinase should be measured when musculoskeletal symptoms are reported; glucose should be monitored when there are risk factors for diabetes. [**2A**]

6.6 All women of child-bearing age should receive pre-pregnancy counselling, with appropriate advice on contraception, before starting a statin and this should be reinforced at annual review. [**2A**]

6.7 Statins and other systemically absorbed lipid regulating drugs should be discontinued 3 months before planned conception, as well as during pregnancy and breast feeding. [**2A**]

6.8 Although carotid ultrasonography has been used in clinical trials, its role in monitoring therapy as part of the clinical care for FH has not been established and it should therefore not be used at present for this purpose. [**3C**]

6.9 Lomitapide and Mipomersen should be considered as adjunctive treatments to diet and cholesterol lowering drugs in adults with homozygous FH to further reduce plasma LDL-cholesterol, particularly if lipoprotein apheresis is not available. [**1C**]

6.10 Well controlled and low complexity patients should be followed-up in primary care, whereas higher complexity patients will need regular review by a specialist, with the option of shared care. Review intervals should vary according to clinical context. Opportunities should be created for integrating the primary and specialist care of FH. [**3B**]

7. Management of Children and Adolescents

7.1 Patients must receive advice on lifestyle modifications and on correcting non-cholesterol risk factors; primordial prevention (counselling to inhibit the development of risk factors) is particularly important. [**2A**]

7.2 To lower elevated plasma LDL-cholesterol in this age group generally requires a fat-modified, heart-healthy diet and a statin, with the possible addition of ezetimibe or a bile acid sequestrant. [**1A**]

7.3 All patients should be treated with diet, with statins considered at age 8 to 10 years and ideally started before age of 18 years; plasma LDL-cholesterol targets in this age group need not be as intense as for

adults [**2B**]

7.4 Boys and girls should generally be treated at similar ages, although with a particularly adverse family history of CHD and other major risk factors, boys with heterozygous FH could be considered for earlier treatment with statins. [**2B**]

7.5 Children, between the ages of 8 and 10 years, with proven FH on a suitable diet and LDL-cholesterol >4.0 mmol/L on two occasions should be started on low-dose statin monotherapy, aiming for an LDL-cholesterol <4.0 mmol/L. [**3C**]

7.6 After the age of 10 years, children with proven FH on a suitable diet and LDL-cholesterol >3.5 mmol/L on two occasions should be started on statin monotherapy, aiming for an LDL-cholesterol <3.5 mmol/L, with the addition of ezetimibe or a bile acid sequestrant if required. [**3C**]

7.7 The preferred statins for initiating therapy are those that are licensed for clinical use in this age group in specific countries; other statins may be prescribed according to clinical indications, higher doses of potent statins being required in homozygotes. [**1C**]

7.8 Although statins can be safely used in children, weight, growth, physical and sexual development, and well-being should be monitored in this age group. [**1A**]

7.9 Plasma levels of hepatic aminotransferases, creatine kinase, glucose and creatinine should be measured before starting drug therapy. All patients on statins should have hepatic aminotransferases monitored; creatine kinase should be measured and compared to pre-treatment levels when musculoskeletal symptoms are reported; glucose should be monitored if there are risk factors for diabetes. [**2A**]

7.10 All adolescent girls should receive pre-pregnancy counselling, with appropriate advice on contraception, before starting a statin and this should be reinforced at annual review. [**3A**].

7.11 Although carotid ultrasonography has been used in clinical trials, its role in monitoring therapy in patients with heterozygous FH has not been established and it should therefore not be used for this purpose. [**3C**]

7.12 Well controlled and lower complexity patients should be followed up in primary care, whereas higher complexity patients will need regular review by a paediatrician. Opportunities should be created for integrated care between GPs and paediatricians. Family based and transitional care clinics should be considered by adult and paediatric services. [**3B**]

7.13 Children with homozygous FH should be referred on diagnosis to a specialist centre and drug and/or apheresis treatment commenced as soon as possible. [**2A**]

7.14 In children with homozygous FH and rapidly progressive atherosclerosis, Lomitapide and Mipomersen, although not yet tested in children, should be considered, employing special access or compassionate use schemes, as adjunctive treatments to diet and conventional drugs to further reduce plasma LDL-cholesterol, particularly if apheresis is not available or declined by the patient/family. [**3C**]

8. Lipoprotein apheresis and related treatments

8.1 Lipoprotein apheresis (LA) should be considered in all patients with homozygous or compound heterozygous FH (i.e. homozygous FH phenotype) and carried out in a dedicated centre with the relevant expertise. [**1A**]

8.2 LA should be considered in patients with heterozygous FH with CHD who cannot achieve LDL-cholesterol targets despite maximal drug therapy or because they cannot tolerate statins. [**2A**]

8.3 LA should be considered in children with homozygous FH by the age of 5 and no later than 8 years. [**2A**]

8.4 Diet and drug therapy to lower LDL-cholesterol should be continued during treatment with LA [**2A**].

8.5 The efficacy, tolerability and safety of LA must be regularly reviewed. [**3A**]

8.6 The effect of LA on progression of atherosclerosis should be monitored according to clinical indications in FH patients with echocardiography (aortic valve and root), carotid ultrasonography and exercise stress testing. [**3B**]

8.7 Lomitapide should be considered as an adjunctive to standard diet and drug therapy to further lower plasma LDL-cholesterol in adults with homozygous FH on LA. [**1C**]

8.8 Lomitapide should be considered, via a special access scheme, as an adjunctive treatment to further lower plasma LDL-cholesterol in children and adolescents with homozygous FH on LA with rapidly progressive atherosclerosis. [**3C**]

8.9 Mipomersen should be considered as an adjunctive to standard diet and drug therapy to further lower plasma LDL-cholesterol in adults, children and adolescents with homozygous FH on LA who cannot tolerate lomitapide. [**3C**]

8.10 If available, orthotopic liver transplantation should be considered for younger patients with homozygous FH who have rapid progression of atherosclerosis or aortic stenosis, cannot tolerate LA or when plasma LDL-cholesterol cannot be adequately lowered with LA, diet and drug treatment. [**3B**]

9. Organization and Development of Care

9.1 Care pathways for FH should be developed for country-specific and local needs. [**3A**]

9.2 Specialist services should be multidisciplinary based and integrated with primary care. [**3B**]

9.3 Specialist care of FH should ideally be supported by cardiology, paediatric, genetic, imaging, transfusion medicine, nursing, dietetic, psychology, pharmacy and pathology laboratory services. [**3A**]

9.4 Cascade screening should ideally be centrally co-ordinated by a dedicated centre. [**1A**]

9.5 Low complexity patients should be managed in primary care, with the option of annual specialist review. [**3A**]

9.6 Higher complexity patients should be managed principally in specialist centres. [**3A**]

9.7 Medical, nursing and allied health staff managing patients with FH should be accredited in cardiovascular prevention. [**3A**]

9.8 Services should establish partnerships with academic and professional organizations to enhance teaching, training and research. [**3A**]

9.9 A registry of patients and families should be established for clinical, research and audit purposes. [**3A**]

9.10 A support group of patients and families should be established as a major priority for enhancing public, government and health care provider awareness, as well as the total quality of care of FH. [**3A**]

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Original Article

Reference Interval for the Apolipoprotein B-48 Concentration in Healthy Japanese Individuals

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Aim: Small intestine-derived chylomicrons and chylomicron remnants, which are predominant in patients with postprandial hypertriglyceridemia, chylomicron syndrome and/or familial dyslipidemia, carry one molecule of apolipoprotein B-48 (apo B-48) per lipoprotein particle. We investigated the reference interval for the apo B-48 concentration.
Methods: We studied 516 individuals who provided written informed consent and confirmed that they were not taking any medications. BMI, waist circumference, blood pressure and the fasting serum concentrations of LDL-C, triglyceride (TG), HDL-C and apo B-48 were measured. The Apo B-48 concentrations were compared according to sex, a pre- or postmenopausal status, dyslipidemia (LDL-C ≥ 140 mg/dL, TG ≥ 150 mg/dL, HDL-C < 40 mg/dL), metabolic syndrome (MetS) and the number of risk factors.
Results: The fasting apo B-48 concentrations (mean ± SD) were significantly higher in men than in women (3.8 ± 3.3 μg/mL vs 2.4 ± 1.9 μg/mL, $p<0.001$), subjects with a BMI of ≥ 25 kg/m^2 versus a BMI of < 25 kg/m^2 (4.4 ± 3.7 μg/mL vs 2.8 ± 2.4 μg/mL, $p<0.001$) and those with versus without MetS (6.5 ± 4.3 μg/mL vs 3.0 ± 2.6 μg/mL, $p<0.001$). High apo B-48 concentrations were also observed in correlation with the number of risk factors for the MetS. The upper reference limit of apo B-48 was estimated to be 5.7 μg/mL among the 332 patients with normolipidemia, excluding those exhibiting a mean value above ± 2.58 standard deviations (SDs), as the mean and range of mean ± 1.96 SD were calculated to be 2.04 μg/mL (reference value) and 0.74 to 5.65 μg/mL (reference interval), respectively.
Conclusions: Based on our study of normolipidemic patients, the upper reference limit for the fasting apo B-48 concentration is estimated to be 5.7 μg/mL.

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Key words: Apolipoprotein B-48 (apo B-48), Chylomicrons, Chylomicron remnants, Reference interval

Introduction

Fasting and postprandial hypertriglyceridemia are serious causative factors of cardiovascular events and sudden cardiac death[1]. An increased serum triglyceride (TG) concentration results from the accumulation of TG-rich lipoproteins (TRLs), particularly after a meal. Postprandial hyperlipidemia refers to the occurrence of a high TG concentration after a meal, which is known to be significantly associated with the development of atherosclerotic cardiovascular disease[2,3]. TRL contain two types of apolipoprotein

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(apo) B, apo B-100 derived from the liver and apo B-48 derived from the small intestine[4].

Chylomicrons (CMs) are synthesized from apo B-48, TG and cholesterol ester in small intestinal cells following the ingestion of lipid-rich foods. After being released into the peripheral blood, CMs are metabolized into smaller remnant particles, CM-remnants, by lipoprotein lipase (LPL) attached to the peripheral vascular wall and taken up by the liver. Apo B-100, a major component of very-low-density lipoprotein (VLDL) is produced in the liver. VLDL is also reduced to smaller VLDL-remnants (or intermediate-density lipoprotein, IDL) by the actions of LPL in the peripheral blood. These remnant particles (CM-remnants and VLDL-remnants) directly infiltrate the vascular wall, subsequently triggering the development of atherosclerotic disease via accelerated macrophage foam cell formation, platelet coagulation and small dense LDL accumulation, as well as the induction of a low concentration of high-density lipoprotein (HDL) cholesterol (HDL-C)[5].

A number of remnant cholesterol assays have been developed and are currently being used to evaluate the risks of atherosclerotic diseases, such as cardiovascular disease (CAD)[6-8]. However, these methods cannot be used to accurately discriminate small intestine-derived CM-remnants from liver-derived VLDL-remnants; therefore, the development of a new assay system is required in order to quantitatively measure the CM-remnant concentration independently. Since one CM-remnant particle contains one apo B-48 molecule and the concentration of apo B-48 is equivalent to that of CM-remnants, we developed a new assay system for measuring the apo B-48 concentration. First, we prepared an enzyme-linked immunosorbent assay (ELISA)[9] for use on a fully automated analyzer system based on the chemiluminescent enzyme immunoassay (CLEIA)[10]. Remnants are usually metabolized immediately; however, the apo B-48 concentration remains elevated due to increased food-derived lipid intake, accelerated TRL synthesis and/or delayed TRL catabolism.

The half-life of the CM particles produced following the ingestion of fat is approximately 30 minutes in the peripheral blood, although the measurable concentration of apo B-48 proteins remains under a fasting condition due to the large amount of lipid absorption and CM production in the small intestine. Therefore, the fasting apoB-48 concentration is correlated with an increase in the TG level following the consumption of a high-fat meal, implying that the fasting apo B-48 concentration is a marker of postprandial hyperlipidemia[11]. High apo B-48 concentrations are usually observed in patients with type III hyperlipidemia[9], metabolic syndrome (MetS)[12], type IIb hyperlipidemia[13] or CD36 deficiency[14]. However, the reference interval for the apo B-48 concentration in healthy fasting individuals has not yet been established.

Aim

In this study, we attempted to establish the upper reference limit and reference interval for the fasting apo B-48 concentration in individuals with normolipidemia.

Subjects and Methods

Subjects

The subjects of this study included 516 individuals who received their annual health checkup and were not taking any medications. The study was carried out under the approval of the Osaka University Health Care Center and Saint (St.) Marguerite Hospital, and all participants provided their written informed consent. The institutional ethics committees of both facilities approved the research protocol. After confirming the lack of a significant adverse medical history known to affect lipoprotein or carbohydrate metabolism, various anthropometric parameters, including height, body weight and waist circumference were obtained and the body mass index (BMI, body weight [kg]/height [m]^2) was calculated. Blood samples were collected in the morning after overnight fasting. The serum samples were then separated via low-speed centrifugation and stocked at −80℃ until the analyses. All specimens were handled according to the protocols of the Helsinki Declaration.

Measurements

Blood pressure (BP) was measured in the sitting position. Hypertension was diagnosed based on a systolic BP of ≥140 mmHg and/or a diastolic BP of ≥90 mmHg. A high BP status was determined based on a systolic BP of ≥130 mmHg and/or a diastolic BP of ≥85 mmHg (according to the guidelines for the management of hypertension issued by the Japanese Society of Hypertension). The serum TG concentration was measured according to an enzymatic method, and the LDL-cholesterol (LDL-C) and HDL-C levels were measured using direct methods. We identified cases of dyslipidemia and normolipidemia based on the diagnostic criteria for dyslipidemia of the Japan Atherosclerosis Society: (a) an LDL-C level of ≥140 mg/dL, (b) a TG level of ≥150 mg/dL, (c) an HDL-C level of

< 40 mg/dL (according to the guidelines for the diagnosis and prevention of atherosclerotic cardiovascular disease for the Japanese)[15]. Abnormal factors were summarized in the patients with dyslipidemia. The fasting plasma glucose (FPG) concentration was measured according to the hexokinase UV method, and the hemoglobin A1c (HbA1c) (JDS) level was measured according to the latex agglutination method. A high fasting glucose level was defined as an FPG of ≥ 110 mg/dL, according to the criteria of the Japan Diabetes Society. MetS was diagnosed based on the criteria of the Japanese Society of Internal Medicine[16], namely, a waist circumference of ≥ 85 cm in men and ≥ 90 cm in women combined with at least two of the following factors: (a) a high BP status and hypertension (a systolic BP of ≥ 130 mmHg and/or a diastolic BP of ≥ 85 mmHg), (b) abnormal lipid metabolism (a TG level of ≥ 150 mg/dL and/or an HDL-C level of < 40 mg/dL), (c) high fasting glucose (an FPG level of ≥ 110 mg/dL). Cardiac risk factors were summarized in cases of MetS.

The serum apo B-48 concentration was determined using the CLEIA system (Fujirebio, Inc., Tokyo, Japan)[10]. Briefly, serum samples were incubated with treatment buffer solution supplemented with surfactant in order to separate apo B-48 from CMs and CM-remnants. The pre-treated samples were incubated with ferrite particles coupled with murine monoclonal antibodies against apo B-48 in the solid phase. After washing, further incubation was carried out with alkaline phosphatase-conjugated anti-apo B monoclonal antibodies as a second antibody. After further washing, a chemiluminescent substrate was added to the test cartridge, after which the relative chemiluminescent intensity was measured and the serum apo B-48 concentration was calculated according to a standard curve.

Statistical Analysis

The statistical analysis was performed using the non-parametric Mann-Whitney *U* test according to F-study with the Stat Flex software program (ver. 6, Artec Inc., Osaka, Japan) after confirming the distribution. The level of significance was assumed to be 95%. The upper reference limit and reference interval for the apo B-48 concentration were estimated according to the methods recommended by CLSI (Clinical and Laboratory Standards Institute). Briefly, after normalizing all data using logarithm conversion, the mean and standard deviation (SD) were calculated and patients exhibiting a mean value above ± 2.58 SD were eliminated. This process was repeated until no exception data were calculated. Subsequently, the value was returned to the integer, and the upper reference limit and reference interval were defined.

Table 1. Clinical and Laboratory Data

	Mean ± SD	95% Confidence Interval
Men/Women	284/232	
Age (year)	42 ± 10/42 ± 11	
Post-menopausal	48/232	
BMI (kg/m^2)	22.4 ± 3.3	22.0-22.7
Waist circ. (cm)	91.1 ± 5.6	90.0-92.2
sBP (mmHg)	115.9 ± 14.6	114.6-117.2
dBP (mmHg)	73.2 ± 11.3	72.2-74.2
TC (mg/dL)	199 ± 31	196.0-201.6
TG (mg/dL)	94 ± 69	87.7-99.7
HDL-C (mg/dL)	65 ± 15	63.1-65.9
LDL-C (mg/dL)	121 ± 29	118.1-123.3
FPG (mg/dL)	87 ± 13	86.2-88.5
HbA1c (JDS) (%)	5.0 ± 0.5	4.9-5.1
	Number of Patients	
BMI ≥ 25 kg/m^2	111 (21.5%)	
BMI < 25 kg/m^2	405 (78.5%)	
Hypertension	47 (9.1%)	
High BP status	103 (20.0%)	
High FPG	10 (1.9%)	
Number of abnormal factors for dyslipidemia		
0	337 (65.3%)	
1	138 (26.7%)	
2	37 (7.2%)	
3	4 (0.8%)	
Number of risk factors for metabolic syndrome (MetS)		
0	303 (58.7%)	
1	135 (26.2%)	
2	53 (10.3%)	
3	24 (4.6%)	
4	1 (0.2%)	

The abbreviations used in this Table are as follows.
dBP: diastolic blood pressure, sBP: systolic blood pressure, BMI: body mass index, FPG: fasting plasma glucose, HbA1c: hemoglobin A1c, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, TC: total cholesterol, TG: triglycerides

Results

Background Characteristics of the Subjects

The total number of registered subjects was 516 (284 men and 232 women: 183 premenopausal patients, 48 postmenopausal patients and one unknown patient) at two hospitals. The assay data and classification of the subjects are summarized in **Table**

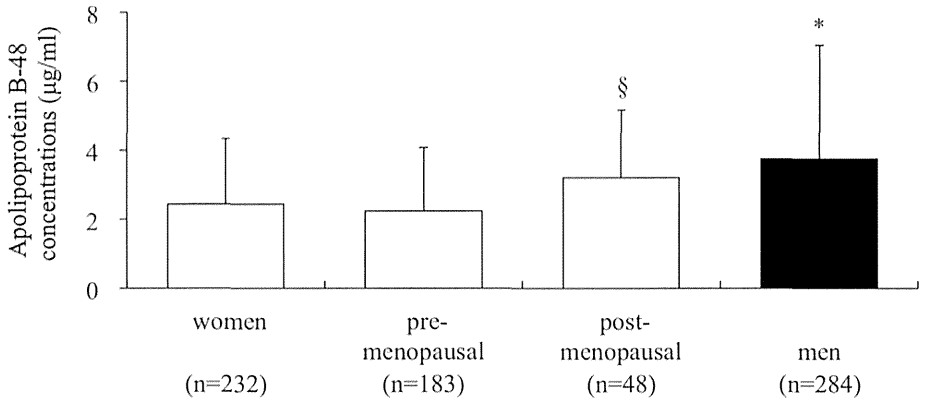

Fig. 1A. Comparison of the apolipoprotein B-48 concentrations in all cases.

The apolipoprotein B-48 concentrations in 284 men and 232 women (183 premenopausal patients, 48 postmenopausal patients and one unknown patient) were compared. The values indicate the mean ± standard deviation as follows: women = 2.4 ± 1.9 μg/mL, premenopausal women = 2.2 ± 1.8 μg/mL, menopausal women= 3.2 ± 2.0 μg/mL, men = 3.8 ± 3.3 μg/mL. Statistical significance was assessed using the Mann-Whitney *U* test. $^{*}p<0.001$ against women, $^{\S}p<0.001$ against premenopausal women.

1. A total of 111 patients had a BMI of ≥ 25 kg/m^2, while a waist circumference beyond the standard range (indicating abdominal obesity) was observed in 114 cases (one-fifth of all cases). Regarding abnormal factors related to dyslipidemia (a high LDL-C concentration, high TG concentration or low HDL-C concentration), two-thirds of the subjects (337 patients, 161 men and 176 women: 152 premenopausal patients and 24 postmenopausal patients) were classified as having no abnormal factors for dyslipidemia; these patients were classified into the normolipidemic group. One-third of the patients exhibited more than one abnormal factor for dyslipidemia. Twenty-four patients, or one-fifth of those with a high BMI (≥ 25 kg/m^2), were diagnosed with MetS, as their waist circumference was beyond the standard range and they exhibited two of three risk factors, including BP, FPG and abnormal lipid metabolism. Most of the patients exhibited either no risk factors (58.7%, 303 patients) or one risk factor (26.2%, 135 patients) for MetS, including hypertension (or a high BP status), hypertriglyceridemia, low HDL-cholesterolemia and a high FPG level.

Apo B-48 Concentrations and their Distribution in Several Classifications

We examined the fasting apo B-48 concentrations after classifying the patients into various groups. First, a sex difference was observed, namely, the mean apo B-48 concentration in men (284 patients) was higher than that observed in women (232 patients)

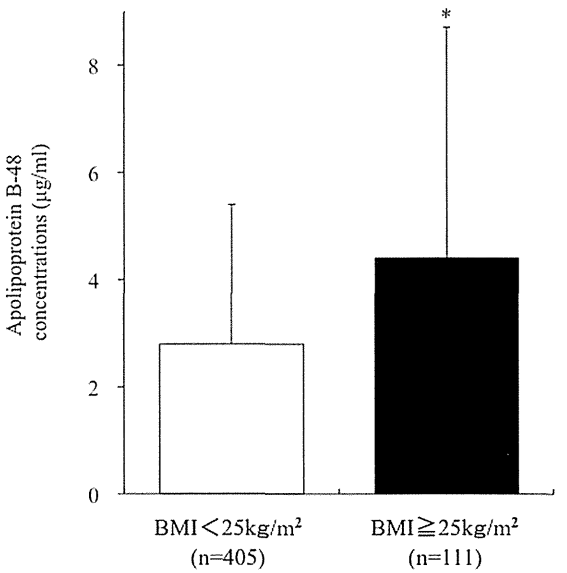

Fig. 1B. Comparison of the apolipoprotein B-48 concentrations in the subjects with a BMI of < 25 kg/m^2 and those with a BMI of ≥ 25 kg/m^2.

The values indicate the mean ± standard deviation, as follows: BMI < 25 kg/m^2 = 2.8 ± 2.4 μg/mL (n=405), BMI ≥ 25 kg/m^2 = 4.4 ± 3.7 μg/mL (n=111). The number of subjects is shown in brackets. Statistical significance was assessed using the Mann-Whitney *U* test. $^{*}p<0.001$

(3.8 ± 3.3 μg/mL vs 2.4 ± 1.9 μg/mL, $p<0.001$, Mann-Whitney *U* test) (**Fig. 1A**). A significant difference was also observed between the pre- and postmenopausal

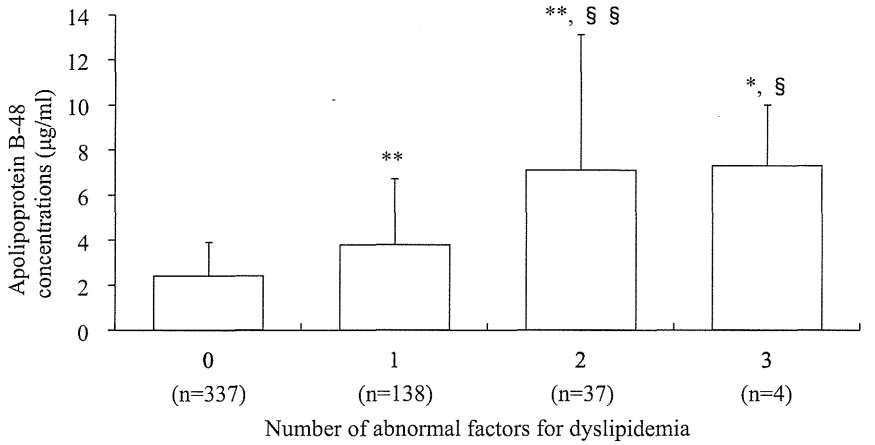

Fig. 2. Comparison of the apolipoprotein B-48 concentrations according to the cumulative number of abnormal factors for dyslipidemia.

The number of abnormal factors for dyslipidemia (a high LDL-C concentration [LDL-C ≥ 140 mg/dL], high TG concentration [TG ≥ 150 mg/dL] or low HDL-C concentration [HDL-C < 40 mg/dL]) was counted in all patients. The apo B-48 concentrations were compared between four groups: patients with no abnormal factors (n=337) and those with one (n=138), two (n=37) and three abnormal factors (n=4). The values indicate the mean ± standard deviation, as follows: no abnormal factors = 2.4 ± 1.5 μg/mL, one abnormal factor = 3.8 ± 2.9 μg/mL, two abnormal factors = 7.1 ± 6.0 μg/mL, three abnormal factors = 7.3 ± 2.7 μg/mL. Statistical significance was assessed using the Mann-Whitney U test. $^{*}p < 0.01$, $^{**}p < 0.001$ against patients with no abnormal factors, $^{\S}p < 0.05$, $^{\S\S}p < 0.001$ against patients with one abnormal factor.

women: the apo B-48 concentrations of the 48 postmenopausal patients were higher than those of the 183 premenopausal patients, while the mean value of the postmenopausal patients was increased, drawing near the average observed in men (3.2 ± 2.0 μg/mL vs 2.2 ± 1.8 μg/mL, $p < 0.001$). When all subjects were classified according to BMI, 111 patients with a BMI of ≥ 25 kg/m^2 were found to exhibit a statistically significantly high apo B-48 concentration in comparison with that observed in the 405 patients with a BMI of < 25 kg/m^2 (4.4 ± 3.7 μg/mL vs 2.8 ± 2.4 μg/mL, $p <$ 0.001, Mann-Whitney U test) (**Fig. 1B**). The number of abnormal factors for dyslipidemia (a high LDL-C concentration [LDL-C ≥ 140 mg/dL], high TG concentration [TG ≥ 150 mg/dL] or low HDL-C concentration [HDL-C < 40 mg/dL]) was counted in all patients. The apo B-48 concentrations in the patients with one (n = 138), two (n = 37) or three (n = 4) abnormal factors for dyslipidemia were significantly higher than those observed in the patients with no abnormal factors for dyslipidemia (n = 337) (**Fig. 2**). The 24 patients with MetS displayed significantly higher apo B-48 concentrations than the 492 patients without MetS (6.5 ± 4.3 μg/mL vs 3.0 ± 2.6 μg/mL, $p < 0.001$, Mann-Whitney U test) (**Fig. 3A**)[16]. In addition, a positive correlation was observed between the apo B-48

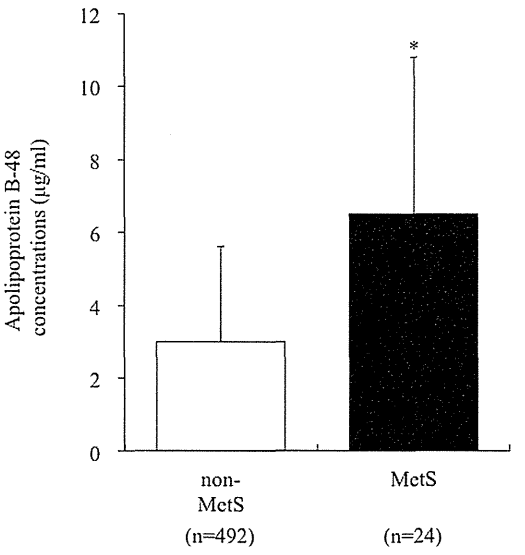

Fig. 3A. Comparison of the apolipoprotein B-48 concentrations in the subjects with or without metabolic syndrome (MetS).

The subjects were divided into two groups, MetS (n=24) and non-MetS (n=492), according to the criteria of the Japanese Society of Internal Medicine. The values indicate the mean ± standard deviation, as follows: non-MetS = 3.0 ± 2.6 μg/mL and MetS = 6.5 ± 4.3 μg/mL. Statistical significance was assessed using the Mann-Whitney U test. $^{*}p < 0.001$

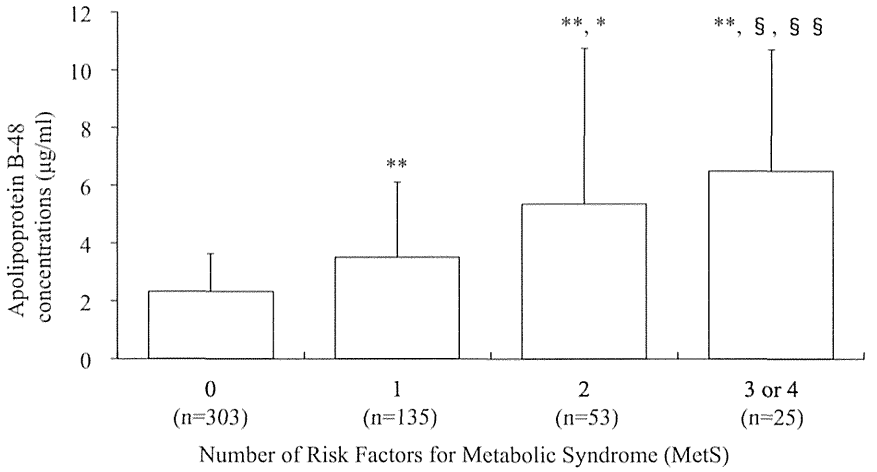

Fig. 3B. Comparison of the apolipoprotein B-48 concentrations according to the cumulative number of risk factors for metabolic syndrome (MetS).

The subjects were divided into four groups: patients with no risk factors ($n=303$) and those with one ($n=135$), two ($n=53$) and three or four risk factors ($n=25$), according to the number of abnormal factors for MetS (waist circumference, a high BP status, high TG/low HDL-C concentrations, a high FPG concentration). The values indicate the mean ± standard deviation, as follows: no risk factors = 2.3 ± 1.3 μg/mL, one risk factor = 3.5 ± 2.6 μg/mL, two risk factors = 5.4 ± 5.4 μg/mL, three or four risk factors = 6.5 ± 4.2 μg/mL. Statistical significance was assessed using the Mann-Whitney U test. $^{**}p<0.001$ against patients with no risk factors, $^{*}p<0.01$, $^{\S}p<0.001$ against patients with one risk factor, $^{\S\S}p<0.05$ against patients with two risk factors.

concentration and the number of risk factors for the components of MetS (hypertension, including a high BP status, hypertriglyceridemia, low HDL-cholesterolemia and a high fasting glucose level) (**Fig. 3B**).

Calculation of the Upper Reference Limit for the Apo B-48 Concentration in the Patients with Normolipidemia

The upper reference limit and reference interval for the apo B-48 concentration were calculated in 337 patients without parameters of abnormal lipid metabolism, as no differences in data were observed between the 152 pre- and 24 postmenopausal normolipidemic patients, as shown in **Fig. 4**; namely, the mean value among the postmenopausal patients increased (2.1 ± 1.2 μg/mL vs 2.6 ± 1.8 μg/mL, not statistically significant) approaching the average observed in the 161 men (2.7 ± 1.7 μg/mL vs 2.6 ± 1.8 μg/mL, not statistically significant). We estimated the upper reference limit for the apo B-48 concentration in 332 normolipidemic patients, excluding those with a mean value of ± 2.58 SD. The calculated mean value and range of mean ± 1.96 SD were 2.04 μg/mL (reference value) and 0.74 to 5.65 μg/mL (reference interval), respectively. Based on these results, we consider 5.7 μg/mL to be the optimum apo B-48 upper reference limit (**Fig. 5**). The reference interval and upper reference limit for the apo B-48 concentration were determined according to the results obtained with the CLEIA system (Fujirebio, Inc., Tokyo, Japan).

Discussion

The occurrence of a high TG concentration after a meal, or postprandial hypertriglyceridemia, is a risk factor for atherosclerosis. Meal-derived TG elevation results from the assembly of CMs, which contain a large quantity of TG in each particle in comparison with VLDL. CMs are immediately hydrolyzed to CM-remnants in patients with normolipidemia, whereas an abnormally high concentration of CM-remnants is observed six hours after meal intake in those with postprandial hypertriglyceridemia. Therefore, the accumulation of CM-remnants due to postprandial hypertriglyceridemia is one of the most serious risk factors for the development of arteriosclerosis-related diseases[17]. Several CM-remnant assay methods have been reported, including the retinyl palmitate method, the combination method employing SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and Western blotting and the remnant-like particle-cholesterol assay method[18]. However, these

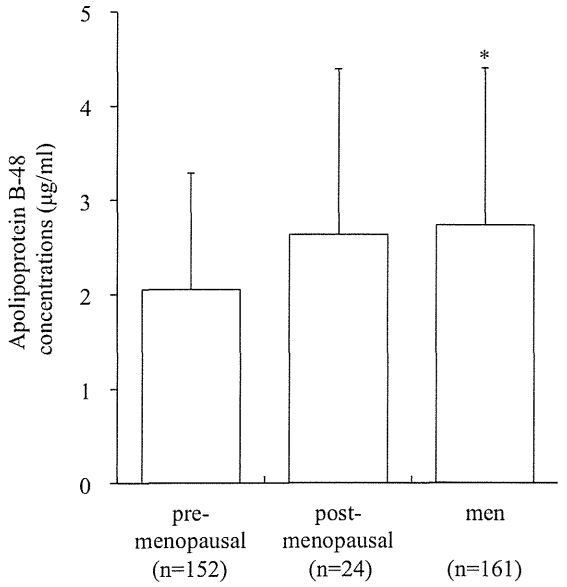

Fig. 4. Comparison of the apolipoprotein B-48 concentrations in the patients with normolipidemia.

The apolipoprotein B-48 concentrations in 161 men and 176 women (152 premenopausal patients and 24 postmenopausal patients) were compared. The values indicate the mean ± standard deviation, as follows: premenopausal = 2.1 ± 1.2 μg/mL, postmenopausal = 2.6 ± 1.8 μg/mL, men = 2.7 ± 1.7 μg/mL. Statistical significance was assessed using the Mann-Whitney *U* test. $^{*}p < 0.001$ against premenopausal women.

methods are associated with problems related to instability, complexity, reproducibility and inaccuracy regarding the assay target[19, 20]. In contrast, apo B-48 is a component of CMs and CM-remnants; therefore, the apo B-48 concentration is a direct marker of alteration of the meal-derived TG concentration, although the apo B-48 concentration in the peripheral blood is approximately one-fiftieth or one-hundredth of the apo B-100 concentration. Several assay methods for measuring the apo B-48 concentration using polyclonal antibodies and/or monoclonal antibodies have been reported[21, 22]. However, as the amino acid sequence of apoB-48 is completely identical to the N-terminal side of apoB-100, it is very difficult to prepare monoclonal and polyclonal antibodies. As a result, the accuracy of these ELISA methods is insufficient for the measurement of apo B-48. On the other hand, an accurate ELISA method was recently developed with the cooperation of Sakai *et al.*[9] using a highly specific monoclonal antibody to the C-terminal of apo B-48 established by Uchida *et al.*[23]. This ELISA system was subsequently improved to create a fully-automated assay system based on CLEIA[10].

In this study, we determined the reference level for the apoB-48 concentration using serum samples obtained from healthy individuals with normolipidemia. Namely, normolipidemic patients were selected by applying the diagnostic criteria for dyslipidemia of the Japan Atherosclerosis Society: (a) an LDL-C level of ≥ 140 mg/dL, (b) a TG level of ≥ 150 mg/dL and (c) an HDL-C level of < 40 mg/dL (Guidelines for the diagnosis and prevention of atherosclerotic cardiovascular disease for the Japanese)[15]. We then used the CLSI recommended method to calculate the reference level. Briefly, we estimated the upper reference limit and reference interval for the apo B-48 concentration in 332 normolipidemic patients, excluding those with a mean value above ± 2.58 SD. We thus determined the reference level for the apo B-48 concentration to be 2.04 μg/mL, the reference interval to range from 0.74 to 5.64 μg/mL and the upper reference limit to be 5.7 μg/mL. Incidentally, a different apo B-48 measuring kit (Human apo B-48 ELISA, Shibayagi, Gunma, Japan) is currently available in Japan. Therefore, the upper reference limit and reference interval for the apo B-48 concentration determined in this study should be restricted to the results obtained using the CLEIA system (Fujirebio, Inc., Tokyo, Japan). We then attempted to determine whether abnormal CM-remnant metabolism was present in the normolipidemia group. When the apo B-48 concentrations of all health checkup patients were measured, a high apo B-48 concentration was observed in the following order: men, postmenopausal women and premenopausal women. The apo B-48 concentrations also differed according to the presence or absence of obesity or MetS. The TG and LDL-C concentrations, which are affected by the apo B-48 concentrations, also differed between men and women and between pre- and postmenopausal women. The upper reference limit and reference interval for the apo B-48 concentration were estimated in patients with normolipidemia; this group also contained patients with hypertension, obesity and hyperglycemia, all of which may affect lipoprotein metabolism. In this study, we examined patients who received their annual health checkup; it was not assumed that these patients had severe metabolic disorders. Therefore, it is necessary to conduct separate studies of different patient groups, including those with relatively severe metabolic disorders.

Recent reports have highlighted the clinical usefulness of the apo B-48 concentration as a screening marker of type Ⅲ hyperlipidemia in patients with accumulated CM-remnants[9, 24] and parameter of the CM-remnants status in those with diabetes mellitus (DM) exhibiting carotid artery plaque[25]. Additionally, correlations have been reported between the apo B-48

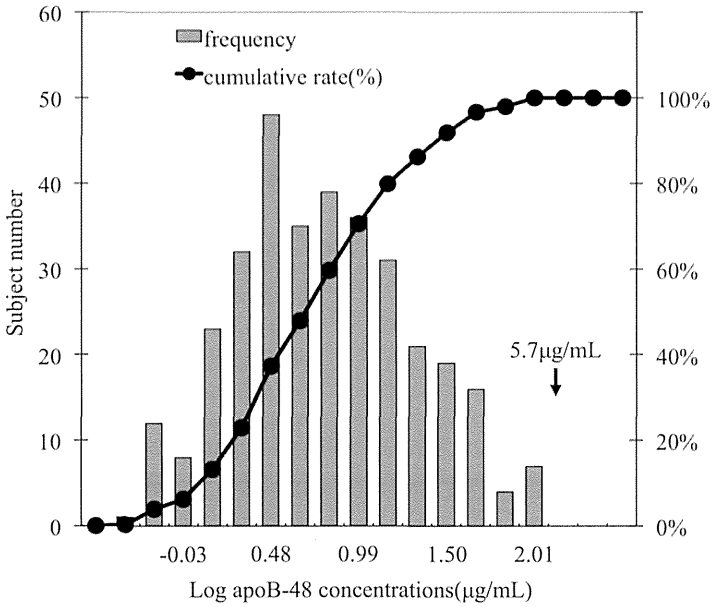

Fig. 5. Distribution of the apolipoprotein B-48 concentrations in the patients with normolipidemia.

The apolipoprotein B-48 concentration is expressed as the log concentration. The upper limit among the 332 patients with normolipidemia was found to be 5.7 μg/mL.

concentration and the carotid intima-media thickness in normotriglyceridemic (100<TG<150 mg/dL) subjects[26] as well as the status of kidney dysfunction in DM patients[27] and the incidence of CAD in ischemic heart disease patients in comparison with other risk factors, such as hypertriglyceridemia, low HDL-cholesterolemia, hypertension and/or hypoadiponectinemia[28]. Furthermore, an elevated incidence of CAD is observed in patients with a high apo B-48 concentration and the risk factors described above. Ultimately, this apo B-48 assay may have numerous applications in future studies.

Conclusion

Based on the results of this multicenter study of Japanese normolipidemic patients not taking any medications, the upper reference limit for the apo B-48 concentration in a fasting state is 5.7 μg/mL, as the mean value was found to be 2.04 μg/mL (reference value) and the mean ± 1.96 SD ranged from 0.74 to 5.65 μg/mL (reference interval).

Study Limitations

The limited number of subjects treated at two clinical facilities likely affected the results of this study.

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Conflicts of Interest

Fujirebio, Inc. shared the costs of apo B-48 measurement. All authors have no other conflicts of interest to disclose.

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